Effect of a Stannous Fluoride Dentifrice on the Sulcular Microbiota: A Prospective Cohort Study in Subjects with Various Levels of Periodontal Inflammation

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Objectives: To assess the effects of an experimental 0.454% stannous fluoride (SnF_2) dentifrice on the oral sulcular microbiota in patients with various stages of oral diseases using checkerboard DNA-DNA hybridization.

Material and Methods: In the present one-month, single center, single product, prospective cohort trial, 37 adults (mean age 37.6) were assigned to one of four oral health condition cohorts with seven to 10 subjects each: 1. mild gingivitis, 2. marked generalized gingivitis to moderate periodontitis, 3. caries-prone and 4. treated moderate to advanced chronic periodontitis in supportive periodontal care.

All four groups were asked to use the test dentifrice and a power toothbrush twice a day for one minute during a four-week test period. Before and after the trial period, Plaque Indices (PII, Silness and Löe, 1964) and Gingival Indices (GI, Löe and Silness, 1963) were recorded. Subgingival plaque samples were collected from all patients at Baseline, as well as after two and four weeks. These samples were analyzed for content of 40 bacterial species using checkerboard DNA-DNA hybridization.

Results: As a result of the only one minute brushing with the stannous fluoride dentifrice, the mean PII at Baseline was significantly lower ($p < 0.05$) from the mean PII at four weeks. No statistically significant differences were found between premolar and molar mean values. Moreover, no statistically significant differences were found between the mean GI at Baseline and at four weeks. The microbiological analysis showed that at baseline subjects in groups 2 and 4 had significantly higher bacterial loads of bacteria than groups 1, and 3 (i.e. *A.actinomyctemcomitans P.gingivalis*, *T.forsythia,* and *T.denticola*. Over the study period, the total bacterial load did not change in groups 2, 3 and 4. In groups 1 and 3, however, an increase in the loads of Streptococci spp. were noticed ($p < 0.05$) including *S.mitis, S.intermedius,* and *S.sanguis* ($p < 0.01$) suggesting an increase in the presence of early colonizing and health associated bacteria.

Conclusion: One minute brushing with a 0.454% stannous fluoride dentifrice did – after four weeks – not affect the subgingival microbial profiles in patients with moderate periodontitis and treated moderate to advanced periodontitis. However, the sulcular microbial profiles of mild gingivitis and caries-prone patients were affected, indicating a shift towards a gingival health associated microbiota in the sulcular region of patients not affected by attachment loss.

Running Head: Effect of stannous fluoride on sulcular microbiota

Key words: biofilm, dentifrice, DNA-DNA hybridization, gingivitis, periodontitis, stannous fluoride

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In the oral cavity, periodontitis and tooth decay are the two predominant bacterial infectious diseases (Marsh and Martin, 1999). Gingivitis is caused by the supragingival bacterial biofilm and is the most commonly encountered periodontal disease. The prevalence of gingivitis in adults fluctuates between 50 to 100% (Stamm, 1986, Schürch and Lang, 2004). Periodontitis with concurrent attachment loss is predominantly associated with an opportunistic bacterial infection including pathogens such as subgingival Gram-negative anaerobic microorganisms, e.g. *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis,* and *Tannerella forsythia* (previously *Bacteroides forsythus*) (Mombelli et al, 1994). A susceptible host leading to an excessive host response is necessary for periodontal tissue destruction (Page and Schroeder 1982, Page 1999).

Since it is difficult to maintain an acceptable level of plaque control on a long-term basis only by using mechanical tooth cleaning (Lindhe et al, 1984), chemical agents have been proposed to offer additional antimicrobial benefits and advocated in applications such as mouthrinses, gels and controlled release devices (Brecx et al, 1992; Tinanoff et al, 1980; 1986; 1989; Mandel, 1988; Caton et al, 1993; DeVore, 1994; Baehni et al, 2003; Clavero et al, 2003; Sekino et al, 2004; Kamagate et al, 2004).

In 1955, the first fluoride-containing toothpaste reaching the US market contained stannous fluoride (Muhler et al, 1955). Toothpaste containing stannous fluoride has been shown to possess anti-plaque, anti-gingivitis, and anti-caries activity on the basis of an antimicrobial action. Stannous fluoride provided some biofilm inhibitory activity, particularly when combined with amine fluoride (AmF). The effects, however, seemed to be due to the non-fluoride ions (Lindhe et al, 2003; Rølla and Ellingsen, 1994). Enamel treated with stannous fluoride became hydrophobic (Rølla et al, 1991). This may inhibit biofilm adhesion (Rølla et al, 1991; Ota et al, 1989; Embleton et al, 2001). Moreover, the antiplaque effect presumably contributed to the antigingivitis effect of stannous fluoride (Rølla and Ellingsen, 1994; Paraskevas et al, 2004; Madlena et al, 2004; Guarnelli et al, 2004; Pizzo et al, 2004). In addition, AmF and SnF_2 seemed to induce an increased oxygen-dependent antibacterial activity of neutrophils *in vitro*, which might contribute to the elimination of bacteria (Shapira et al, 1997). A new dentifrice containing stabilized 0.454% stannous fluoride has been shown to significantly reduce gingivitis, gingival bleeding and supragingival plaque (Archila et al, 2004; Mankodi et al, 2005). This is consistent with another study (Beiswanger et al, 1995). In addition, stannous fluoride may have a direct effect on the activity of dental biofilm metabolism (Wu and Savitt, 2002). A few microbiological analyses have shown significant reductions in *Streptococcus mutans* counts (Klock et al, 1985; Tinanoff et al, 1989). Other studies did not reveal any adverse shifts among oral microbial populations or overgrowth of opportunistic pathogens within a bacterial ecosystem. Moreover, no oral microbial resistance to SnF_2 has been detected (Wu and Savitt 2002).

Thus, new compositions with stabilized stannous fluoride appear to reduce the inherent instability of the stannous ion, reviving the significance of the agent in providing an important adjunct to the prevention and control of gingivitis.

A cost-effective microbiological identification method using a checkerboard DNA-DNA hybridization has been developed (Socransky et al, 1994; 2004). DNA probes are suitable to identify and enumerate bacterial species in complex communities such as dental plaque biofilms. Thus, the method permits the simultaneous determination of the presence of 40 bacterial species (Ximénez-Fyvie et al, 2000). Consequently, therapeutic effects can be examined and results from such analysis can indicate whether the target species of the microbiota were affected by intervention (Socransky et al, 2004).

The aim of the present study was to use a prospective cohort study design using the baseline conditions as the control to evaluate the effect of an experimental 0.454% stannous fluoride dentifrice on the microbial complexes in patient cohorts of varying oral health conditions, ranging from mild gingivitis to periodontitis severity. The checkerboard DNA-DNA hybridization method was to be used for analysis fo the sulcular microbiota.

MATERIALS AND METHODS

In total, 38 patients (19 female, 19 male) were recruited from the patient pool of the Department of Periodontology and Fixed Prosthodontics of the School of Dental Medicine, University of Berne. All participants were healthy individuals, 23-62 years old, who had at least 20 teeth presented.

The study was composed of four cohorts of seven to 10 subjects each. The four cohorts were to represent various stages of periodontal pathology and risk for disease progression. *Group 1* consisted of patients displaying localized and mild gingivitis. *Group 2* yielded marked generalized gingivitis in conjunction with moderate chronic periodontitis. These patients were recruited after the completion of the initial (hygienic) phase of periodontal therapy, but prior to the surgical therapy of residual pockets. *Group 3* contained patients classified as periodontally healthy or, at most, showing gingivitis, but with a history of dental caries. *Group 4* consisted of recall patients with a history of successfully treated periodontitis. All participants were informed about the outline, the purpose and the duration of the study and signed a consent form. The study was approved by the Cantonal Ethical Committee (KEK) of the Canton of Berne, Switzerland.

During the duration of four weeks, all the patients agreed to refrain from using any other dentifrice or oral rinse than the products delivered by the study organization.

The dentifrice used in the study contained 0.454% stannous fluoride (Procter & Gamble Company, Cincinnati, Ohio, USA). Dentifrice tubes were labelled with a single panel clinical test label containing the study number, subject number, usage instructions, caution statements, net contents, and other information as dictated by internal regulatory requirements and clinical standard operating procedures. In addition, each subject was issued an electrical Crest Spinbrush Pro™ (Procter & Gamble Company, Cincinnati, Ohio, USA).

A four-week prospective cohort study was performed. At Baseline, a test kit containing one tube of the experimental dentifrice, one Crest Spinbrush Pro™ toothbrush, a 60-second timer and an instruction sheet was provided. The subjects were asked to use the experimental dentifrice twice a day, one minute each time, and using their customary cleaning technique for the four-week test period. No efforts in changing the oral hygiene habits were made.

Patients with a history of any medical diseases and/or therapies that might have interfered with the study were not included. In addition, none of the patients had taken antibiotics within seven days of the baseline examination. The outline of the study is depicted in Table 1.

Table 1 Study design

Weeks	0	1	2	3	4
Examination	Baseline		2		3
Plaque Index	X				X
Gingival Index	X				X
Plaque sampling	X		X		X

At Baseline, after two and four weeks, the clinical examinations were performed by the same calibrated examiner (B.P.). For all teeth, except the third molars, the Plaque Index (Silness and Löe, 1964) and the Gingival Index (Löe and Silness 1963) were recorded at six sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual, mesiolingual).

Subgingival (sulcular) plaque samples were taken at Baseline, after two and four weeks and analyzed using the checkerboard DNA-DNA hybridization technique (Socransky et al, 1998). In groups 2 and 4, subgingival plaque samples were also collected in a subset of residual periodontal pockets.

After drying and isolation with cotton rolls, the samples were taken using a sterilized Gracey curette 11/12 or 13/14 (Hu-Friedy, Leimen, FRG). At each visit the same tooth surfaces were studied. Two different Gracey curettes were used to obtain subgingival biofilm, one for mesiobuccal of first molars in each quadrant and one for the mesiobuccal of first premolar in each quadrant.

Pooling of plaque samples was performed for molars and premolars. The samples were immediately suspended in two sterile Eppendorf tubes containing 600 µl of TE buffer and mixed with 400 µl of a Sodium Hydroxide solution. The plaque samples were then placed in –20°C until the analysis was performed in the laboratory.

Counts of 40 subgingival species were determined in each plaque sample, using a modification (Haffajee et al, 1997) of the checkerboard DNA-DNA hybridization technique (Socransky et al, 1994). The samples were lysed, and the DNA placed in lanes on a nylon membrane using a Minislot device (Immunetics, Cambridge MA). After fixation of the DNA to the membrane, the membranes were placed in a Miniblotter 45 (Immunetics, Cambridge MA) with the lanes of DNA at 90° to the lanes of the device. Digoxigenin-labeled whole genomic DNA probes to 40 subgingival bacterial

Table 2 Plaque Index (PII: mean ± SD) for each group at Baseline and post-treatment

Group	N	Baseline			4 weeks		
		Premolar Mean ± SD	Molar Mean ± SD	Full-mouth Mean ± SD	Premolar Mean ± SD	Molar Mean ± SD	Full-mouth Mean ± SD
1	10	0.1 ± 0.1	0.2 ± 0.2	0.3 ± 0.2	0.4 ± 0.4*	0.5 ± 0.4*	0.4 ± 0.2*
2	7	0.1 ± 0.1	0 ± 0	0.1 ± 0.1	0.6 ± 0.8*	0.6 ± 0.5*	0.5 ± 0.6*
3	10	0.2 ± 0.3	0.1 ± 0.2	0.2 ± 0.1	0.4 ± 0.3	0.4 ± 0.3*	0.4 ± 0.2*
4	10	0.2 ± 0.2	0.2 ± 0.2	0.3 ± 0.2	0.8 ± 0.6*	0.8 ± 0.7*	0.7 ± 0.6*

* p = 0.01

species were hybridized in individual lanes of the Miniblotter. After hybridization, the membranes were washed at high stringency and the DNA probes detected using antibody to digoxigenin conjugated with alkaline phosphatase and chemifluorescence detection. The probes and their source strains were described in Ximenez-Fyvie et al (2000). Signals were detected using AttoPhos substrate (Amersham Life Science, Arlington Heights, Illinois, USA) and a Storm Fluorimager (Molecular Dynamics, Sunnivale, CA, USA). Two lanes in each run contained standards at concentrations of 10^5 and 10^5 cells of each species. The sensitivity of the assay was adjusted to permit detection of 10^4 cells of a given species by adjusting the concentration of each DNA probe. Signals were evaluated using the Storm Fluorimager and converted to absolute counts by comparison with the standards on the same membrane.

The bacterial load of individual pathogens were automatically defined by the Storm Fluorimager and its software program. The bacterial load by complex was computed as the sum of each individual bacterium assessed. The total bacterial load was computed as the sum of the bacterial load by each bacterium assessed. Thus, the data analysis is compromised to the panel of 40 species and not reflecting the bacterial load of other bacteria not recognized by the checkerboard assay.

The data were analyzed using one-way ANOVA and by nonparametric test methods (Mann-Whitney U-Test), with a level of significance set at $\alpha = 0.05$.

RESULTS

Clinical assessment

In the present study, complete data were obtained for 37 subjects. One subject from Group 1 dropped out after five days because of a desquamation of the oral tissues and a change in taste sensation. Group 1 consisted of five females and five males, with a mean age of 24.3 (SD 1.3) years. Group 2 consisted of three females and four males, with a mean age of 49.7 (SD 9.4) years. Group 3 contained eight females and two males, with a mean age of 25.9 (SD 5.7) years. Group 4 consisted of two females and eight males, with an age of 52.8 (SD 7.7) years.

Descriptive statistics of premolar, molar and full-mouth Plaque (PII) and Gingival indices (GI) at Baseline and after four weeks are summarized in Tables 2 and 3, respectively. The mean premolar PII and the mean molar PII did not differ significantly between Baseline and at four weeks (Table 2). However, the full-mouth PII between Baseline and 4 week-examination increased significantly for all four groups ($p < 0.05$, Mann-Whitney U-tests). Statistical analysis failed to demonstrate significant differences in gingival assessments between Baseline and at week 4. (Table 3).

The mean Gingival Index (GI) scores for each group at Baseline and post- treatment are presented (Table 3). At the beginning of the study, all participants revealed the mean full-mouth GI = 1.1 ± 0.3. After four weeks, the mean full-mouth GI was 1.2 ± 0.3. There was no statistically significant difference between Baseline and 4 weeks mean GI.

Table 3 Gingival Index (GI:mean ± SD) for each group at Baseline and the four week examination

Group	N	Baseline			Endpoint		
		Premolar Mean ± SD	Molar Mean ± SD	Full mouth Mean ± SD	Premolar Mean ± SD	Molar Mean ± SD	Full mouth Mean ± SD
1	10	1.0 ± 0.3	1.0 ± 0.2	1.1 ± 0.3	1.0 ± 0.4	1.2 ± 0.3	1.2 ± 0.3
2	7	1.2 ± 0.2	1.2 ± 0.2	1.3 ± 0.1	1.1 ± 0.2	1.2 ± 0.4	1.2 ± 0.3
3	10	1.1 ± 0.4	1.1 ± 0.5	1.2 ± 0.4	1.1 ± 0.5	1.1 ± 0.5	1.2 ± 0.5
4	10	1.1 ± 0.4	1.3 ± 0.6	1.2 ± 0.4	1.3 ± 0.2	1.4 ± 0.4	1.3 ± 0.2

Microbiological Assessment

Fig 1 presents an example of the checkerboard DNA-DNA hybridization used to detect 40 bacterial species with presumptive pathogenic potential for periodontitis. The vertical lanes contained the plaque samples of 28 subject samples. Notice that the two vertical lanes at the far right constitutes the reference standards for each bacterium. The horizontal lanes contained the DNA probes in a hybridization buffer for the 40 species studied. A signal at the intersection of the vertical and horizontal lanes indicated the presence of a specific bacterial species and was automatically calculated by the software program.

A comparison of Baseline DNA counts (x10^5) of the 40 bacterial species in the groups without a history of chronic periodontitis (Groups 1 and 3), and in the groups with such a history (Groups 2 and 4) a significantly higher DNA count (x10^5) for *V. parvula, S. gordonii, S. mitis, S. sanguis, A. actinomycetemcomitans, C. ochracea, C. sputigena, C. gracilis, C. showae, F. nucleatum* ss. *nucleatum, F. nucleatum ss. vincentii, T. forsythia, P. gingivalis, T. denticola, N. mucosa, P. melaninogenica, T. socranskii* was detected in Groups 2 and 4. Moreover, higher bacterial DNA counts (x10^5) were detected in the groups without a history of chronic periodontitis compared to Groups 2 and 4 for *E. corrodens, L. buccalis and S. anginosus.*

The total bacterial DNA (x10^5) in groups 2, 3, and 4 did not change over time. In group 1, however, the total bacterial load increased ($p < 0.05$). This change was attributed to an increase of bacteria in the yellow complex during the cause of the study (Streptococci species) (Figure 2). In spite of no significant differences in total bacterial load for group 3, a similar change for the yellow complex was also found ($p < 0.05$). These differences were predominantly attributed to the levels of *S.mitis* ($p < 0.01$), and to *S. intermedius* ($p < 0.01$).

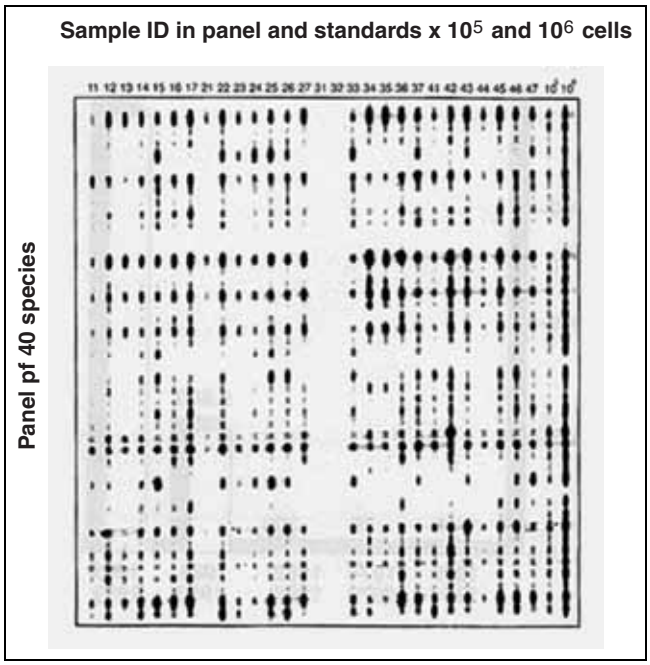

Fig 1 Example of bacterial identification using checkerboard DNA-DNA hybridization for 40 periodontal bacterial species.

In Group 1 (mild gingivitis), the profile of the bacterial species that changed significantly are presented (Figure 3). Between Baseline and 4 weeks post- treatment, statistically significant increases ($p < 0.01$) were found for *S.mitis, S.intermedius, S.anginosus*). In addition the levels of *V.parvula, C.gracilis, C.showae, F.periodonticum, N mucosa, P. melaninogenica*, and *T.socranskii* also increased ($p < 0.05$). In group 3 statistically significant in-

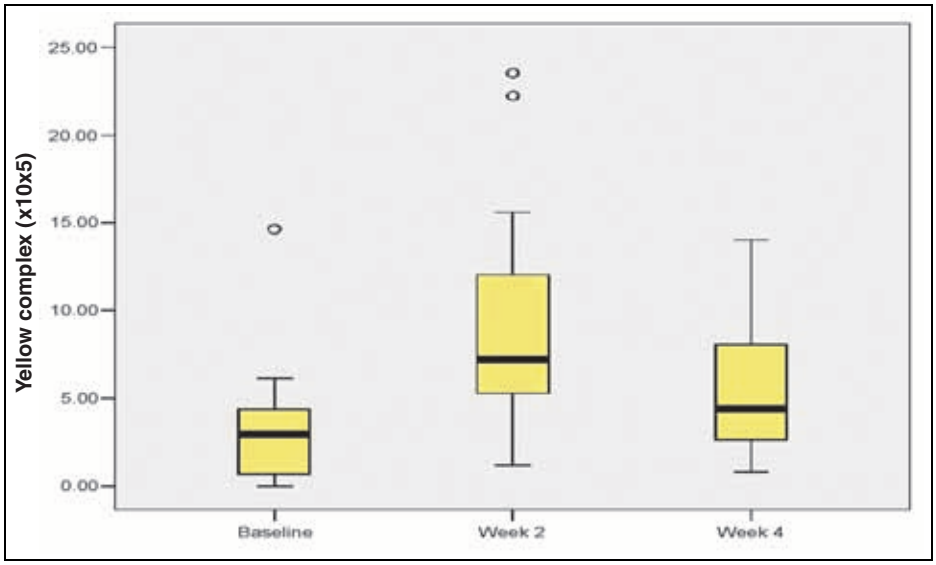

Fig 2 Baseline, Week 2 and Week 4 bacterial load in Group 1 (mild gingivitis) and for the yellow complex.

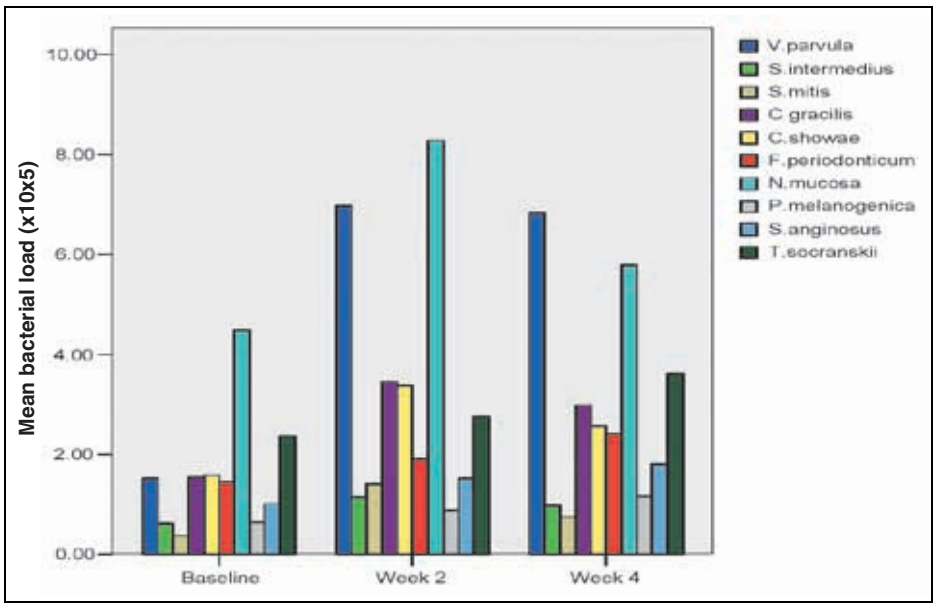

Fig 3 Mean values of bacterial load for bacteria that changed significantly over time in Group 1 (mild gingivitis).

creases ($p < 0.01$) were found for *S.mitis, S.intermedius,* and *S.sanguis* (Figure 4). In group 2 (marked gingivitis and chronic periodontitis) and in group 4 (successfully treated patients in recall) statistical analysis failed to demonstrate changes over time (data not illustrated).

Further analysis demonstrated that, at baseline, statistically significant higher bacterial loads were found for 22/40 species including *V.parvula* ($p < 0.01$), four Streptococci species, *A.actinomycetemcomitans* ($p < 0.05$), *T.forsythia* ($p < 0.001$), *P.gingivalis* ($p < 0.001$), *T.denticola* ($p < 0.001$), *T.socranskii* ($p < 0.01$), *N.mucosa* ($p < 0.001$), *L. buccalis*, ($p < 0.02$), *S.anginosus* ($p < 0.02$), and *P.melaniogenica* ($p < 0.02$). At Week 2, those differences remained for for 15/40 species including *S.oralis, S.anginosus*, and *T.socranskii*, but were reduced for *T.forsythia* ($p < 0.02$) *P.gingivalis* ($p < 0.02$) and *T.denticola* ($p < 0.02$). It was noticeable that the difference for *A.actinomycetemcomitans* disappeared and remained at week 4. Thus, at week 4 there was a higher bacterial load in groups 2 and 4 combined for 19/40 species studies. These differences were again most noticeable for the bacterial load of *T.forsyhtia* ($p < 0,001$), *P.gingivalis* ($p < 0.001$), and *S.anginosus* ($p < 0.02$).

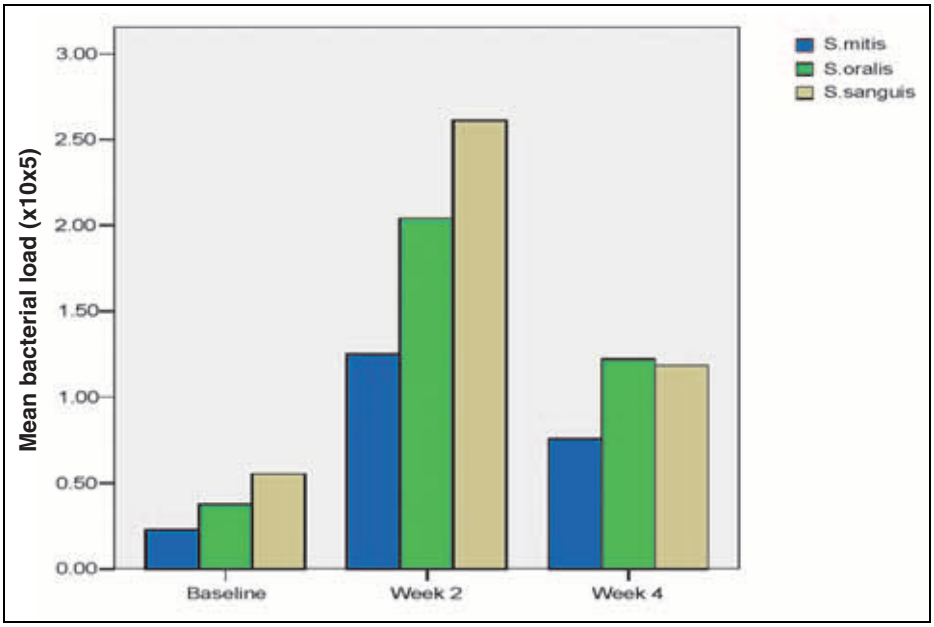

Fig 4 Mean values of bacterial load for bacteria that changed significantly over time in Group 3 (periodontally healthy, some evidence of gingivitis but with a history of caries).

DISCUSSION

Daily removal of biofilm is indispensable for establishing stable periodontal conditions. Biofilms accumulate along the gingival margin and other niches which may be difficult to mechanically clean. There is evidence, however, that even regular mechanical removal of the biofilm may be incomplete in the majority of the population. Motivation, skills and dexterity of the patient are important prerequisites for effective biofilm removal as well (Lindhe and Koch, 1967). Hence, supplemental methods for improving the control of the biofilm may be welcome.

In that respect, the supplemental application of stannous ions (Sn_2+) with their antibacterial activity superiority to that of the stannic ions (Sn_4+) may provide an efficacious principle for the control of biofilm formation. Many studies have examined the clinical efficacy of stannous fluoride gels, mouthrinses and dentifrices with respect to dental caries and gingivitis prevention (Svatun et al, 1978; Bay and Rølla, 1980; Klock et al, 1985; Stamm, 1986; Tinanoff et al, 1989; Boyd et al, 1994). Mixed results have been reported concerning the effects of SnF_2 on biofilm formation and gingivitis. However, until now, the effects of SnF_2 on subgingival biofilm pathogens have not been studied.

The present study was designed to test the effects on the residual biofilm deposits and gingival inflammation, and the subgingival microbiota of a stannous fluoride supplemental to routine mechanical plaque removal. For this purpose, four different patient cohorts were recruited to represent subjects with various histories and exposures to periodontal conditions and caries.

In all of the cohorts the Plaque indices were negatively affected by the four-week use of the stannous fluoride dentifrice. This may partly be due to the fact that the subjects were allowed to brush their teeth only for a standardized and limited period of time. Thus, the study protocol using a supplemental stannous fluoride failed to compensate for the short one minute tooth brushing protocol.

Despite of the increased PII, however, the mean Gingival indices remained unchanged. Thus, it is possible that stannous fluoride dentifrice expresses some anti-inflammatory effect. Although not detected clinically by the plaque index the microbiological results suggested some limited improvement in the profile of microbiota suggesting that, short term, SnF_2 influenced the microbial composition of the biofilm resulting in a less pathogenic microbiota after two weeks.

The data suggested that the supplemental use of the stannous fluoride toothpaste had an effect on the sulcular microbiota. As expected, higher bacterial load for several of the putative periodontal pathogens were found in groups 2 and 4,

(Socransky et al, 1994; 1998; 2004). It is of importance to notice that the levels of *S.sanguis*, and *S.mitis* increased with the tooth paste in groups 1 and 3. These bacteria have been associated with early colonization and development of a biofilm. However, these changes did not exceed half a logarithm and hence, it may be postulated that resistance of bacteria to the SnF_2 did not occur.

As expected, the data of the present study indicate that the total bacterial DNA load in the sulcular(subgingival) plaque samples differed between subjects with various periodontal conditions. The 40 bacterial species identified by the DNA-DNA checkerboard method (Socransky et al, 1998) could be detected in all subjects examined. However, major differences in the composition of the biofilms were found between subjects with no history of periodontitis (i.e. gingivitis or dental caries experience) and those with such a history (i.e. periodontitis or supportive periodontal therapy groups).

The total bacterial DNA in the orange, yellow, purple, green and other complexes (Ximénez-Fyvie et al, 2000) from the mild gingivitis subjects yielded a statistically significant increase after four weeks of application of the stannous fluoride dentifrice. In addition, dental caries subjects demonstrated a significant increase in the total bacterial DNA of the yellow complex after four weeks. As for the other complexes, there weren't any statistically significant differences between Baseline and the four weeks examination in the dental caries cohort. This, in turn, may indicate that – whenever probing depths have not yet increased beyond the three milimeter sulcus- a four week application of stannous fluoride may induce some shifts in the sub-gingival microbiota towards more periodontal health associated bacterial complexes.

However, such shifts in the microbial complexes were not observed in the patient cohorts with an experience of periodontitis (i.e. periodontitis and supportive periodontal therapy subjects), since no differences in the total bacterial DNA load were identified for any of the bacterial complexes between Baseline and the four- week examination. It is most likely that the application of a dentifrice may not affect the subgingival environment of periodontal pockets and hence, only a limited effect on the subgingival microbiota can be expected in subjects with residual periodontal pockets of a four week application of stannous fluoride.

One important observation of the present study is that both at baseline and at week 4, the subjects who clinically had been successfully treated for periodontitis and were on a supportive periodontal care program had significantly more total bacterial DNA than any of the other cohorts. This may indicate that, indeed, periodontal supportive therapy using a routine recall protocol and, in this case, with adjunctive use of a stannous fluoride toothpaste could not be maintained with a microbiota consistent with periodontal health.

The comparison of total bacterial DNA between molar and premolar sites showed no statistically significant differences at Baseline, after two and four weeks. Therefore, it may be assumed that, in these groups of subjects without overt pathology, subgingival biofilm samples obtained either from molar or premolar sites may be representative for the individual's dentition.

Although there was no decrease of the total bacterial DNA load in all four groups, this study has shown that, even if the PII are higher at the endpoint in all four groups, the GI as well as the total DNA of the 40 pathogens in the collected sites had stayed the same. Therefore, there was a negative change of the quantity of biofilm (increase), but a positive change of the quality of the biofilm (fewer pathogens).

The checkerboard DNA-DNA hybridization technique used to identify the various bacterial complexes in the present study does have limitations: 1) only species for which DNA probes have been prepared can be detected. Hence, additional pathogens identified in culture would not be detected by this method. 2) The use of entire genome of the bacterial strain might increase the probability of cross-reactions between species because of common regions of DNA among closely related species. However, cross-reactions between heterogeneous species seem to occur in less than the 1% threshold employed in the technique used (Socransky et al, 2004). Therefore, such reactions can be neglected for the analysis of the present data set. It should, however, be noticed that the mean values for various bacteria and at different time points and diagnostic groups, in principle, remained at levels above a suggested 1×10^5 detection level.

CONCLUSIONS

Within the limits of this study, the results of the present short-term clinical trial have demonstrated that a twice a day, one-minute application of SnF_2

contained in a dentifrice does not seem to affect the composition of the subgingival microbiota in subjects with a history of periodontitis (chronic periodontitis or in supportive periodontal care). However, in subjects with no periodontal pockets and – at the most- mild gingivitis or in caries-prone subjects, the subgingival microbiota shifted for several bacterial complexes towards a microbiota associated with gingival health, and that of early plaque colonization.

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